heme oxygenase-1 (HO-1) pathway, which is able to counteract cellular stress *in vitro* and *in vivo*. The present study was aimed at identifying a possible regulatory effect of HO-1 on HMGB1 in OA synoviocytes.
Methods: Synovial tissue samples were obtained from 15 OA patients undergoing total knee joint replacement. Synoviocytes (fibroblasts and macrophage-like) were obtained by digestion with collagenase IA, cultured in third passage and treated with interleukin-1β (Il-1β, 100 U/ml) for 24 h. HO-1 was induced by treatment with cobalt protoporphyrin IX (CoPP, 10 μM). Matrix metalloproteinase (MMP) activity was determined by fluorometric procedures and HMGB1 release by ELISA. Protein expression was studied by Western blot.
Results: Basal expression of HMGB1 protein was reduced by HO-1 induction in OA synoviocytes. In addition, HMGB1 release into the medium was significantly decreased. Stimulation of synoviocytes with Il-1β resulted in an enhancement of HMGB1 cellular content and release. Our results indicate that both processes are down-regulated by HO-1 overexpression. In addition, HO-1 reduced RAGE expression in these cells. The effects of HO-1 induction were prevented when synoviocytes were transfected with a siRNA specific for human HO-1. In cells without HO-1 induction, HO-1 gene silencing resulted in the up-regulation of HMGB1 and RAGE. Regulation of HMGB1 by HO-1 was accompanied by the inhibition of MMP activity in synoviocytes stimulated with Il-1β.
Conclusions: Our data provide evidence that HO-1 can regulate HMGB1 in OA synoviocytes. Overall, HO-1 signaling appears to be an appropriate target for the development of novel therapies affecting articular disorders.

436 EFFECT OF EPIGALLOCATECHIN GALLATE ON THE INFLAMMATORY RESPONSE OF Il-1-EXPOSED SYNOVIAL FIBROBLASTS

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Purpose: Inflammation is increasingly recognized as contributing to the symptoms and progression of osteoarthritis (OA). Synovitis is a factor that likely contributes to dysregulation of chondrocyte function, favoring an imbalance between the catabolic and anabolic activities of the chondrocyte in remodeling the cartilage. In recent years, significant interest has emerged in the beneficial health effects attributed to the green tea polyphenols. Polyphenols in green tea are potent antioxidants, with the majority of the beneficial effects elicited by epigallocatechin-3-gallate (EGCG), one of the main constituent of green tea.
Among several inflammatory mediators, interleukin-1beta (Il-1) plays a pivotal role in the pathophysiology of OA. We therefore assessed the effect of EGCG on the production of interleukin-8 (Il-8) and interleukin-1 receptor antagonist (Il-1ra) in primary cultured synovial fibroblasts stimulated with Il-1.
Methods: Human synovial fibroblasts were stimulated for 24 hours with Il-1 (10 ng/ml) in the presence or absence of EGCG (0.1−5 μM).
The levels of Il-8 and Il-1ra were measured in cell supernatants by enzyme-linked immunoassay methods. The lack of cell cytotoxicity of EGCG was ensured using the colorimetric MTT assay.
Results: Treatment of synovial fibroblasts with EGCG resulted in a marked inhibition of Il-1-induced Il-8 production. EGCG also increased in a dose-dependent manner the release of Il-1ra by stimulated fibroblasts.
Conclusions: The present study shows that EGCG, at dose comparable with plasma concentration achieved by the consumption of two cups of tea, suppressed the inflammatory response of Il-1-exposed synovial fibroblasts. The suppressive effect of EGCG may be due to the interference with inflammatory signal transduction pathway or may be related to the inhibition of the release and the accumulation of reactive oxygen species. Our findings suggest that EGCG may be of potential therapeutic value in the treatment of OA.

437 EFFECT OF EPIGALLOCATECHIN GALLATE ON CALCIUM CRYSTAL-INDUCED CHEMOTACTIC FACTORS

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Purpose: Although osteoarthritis (OA) is defined as a cartilage disease, synovitis involving mononuclear cell infiltration and overexpression of proinflammatory mediators is common in early and late OA. Calcium crystals deposition is a factor that likely contributes to synovial membrane inflammation.
Polyphenols in green tea are potent antioxidants, with the majority of the beneficial effects elicited by epigallocatechin-3-gallate (EGCG), one of the main constituent of green tea. The aim of our study was to evaluate if EGCG may influence some inflammatory aspects of OA. To this aim we studied the effect of EGCG on chemotactic factors released by human fibroblasts stimulated with calcium crystals, regular features of the most severe forms of OA.
Methods: Human synovial fibroblasts were stimulated with pyrophosphate dihydrate (CPPD) and basic calcium phosphate (BCP) crystals (0.01−0.1 mg/ml) in the presence or absence of EGCG (0.1−5 μM). Il-1beta (10 ng/ml) was used as a positive control.
CPPD and BCP crystals were synthesized by the methods of Cheng and McCarthy respectively. The levels of MCP-1 were measured in cell supernatants by enzyme-linked immunoassay methods. The chemotactic effect of culture supernatants was evaluated on chemotaxis chamber by the migration of fresh-isolated mononuclear blood cells. The lack of cell cytotoxicity of both EGCG and calcium crystals was ensured using the colorimetric MTT assay.
Results: EGCG inhibited MCP-1 release by stimulated fibroblasts in a dose-dependent manner. Supernatants of crystals-stimulated cells loose their ability to induce mononuclear cell migration when EGCG was added in the medium. EGCG inhibited both MCP-1 release and supernatants chemotactic activity of Il-1beta stimulated culture in a dose-dependent manner.
Conclusions: The present study shows that EGCG, at dose comparable with plasma concentration achieved by the consumption of two cups of tea, modify the inflammatory response of calcium crystal-exposed synovial fibroblasts. EGCG may interfere with inflammatory signal transduction pathway and may also inhibits the cellular generation, the release and the accumulation of reactive oxygen species. Our results suggest that EGCG might represents a good candidate for the prevention and treatment of OA.

438 MODULATION OF THE INFLAMMATORY AND CATABOLIC RESPONSE BY PROSTAGLANDIN E2 (PGE2) IS PARTIALLY DEPENDENT ON THE INDUCTION OF DUAL SPECIFICITY PHOSPHATASE 1 (DUSP-1) IN VITRO AND IN VIVO

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Purpose: Prostaglandin E2 (PGE2) is an eicosanoid with pleiotropic properties that binds G-protein coupled receptors, effecting changes in cell signalling through activation of protein kinases and phosphatases. We examined PGE2-dependent control of the dual specificity phosphatase-1 (DUSP-1) in arthritis-affected human synovial fibroblasts (HSF) in culture and in the dorsal air-pouch mouse model of synovial inflammation in vivo.
Methods:
- Cultured human synovial fibroblasts (HSF) were obtained by sequential enzymic digestion from osteoarthritis-affected synovial membranes.
- Western and Northern blot analyses were used to measure protein, phosphorylated protein and mRNA expression, respectively.
- Transient transfection assays were employed to express activated signaling molecules, shRNA constructs, and to analyze reporter luciferase activity.
- Wild type and DUSP-1 null mice were used in the dorsal air pouch studies. Analytes were measured by ELISA and RT-PCR. Cell infiltration measurements were assessed by flow cytometry.
- Statistical analyses included Student's T-test and ANOVA

Results: PGE2 induced a robust (7 fold) and rapid (10 min) increase in DUSP-1 mRNA in cultured HSF, reaching a zenith at 30−60 min followed by decay to control levels. A late phase of DUSP-1 mRNA expression was observed after 4 h and continued for another 20 h. In transient transfection assays using a DUSP-1 promoter-luciferase reporter construct or a luciferase reporter construct harbouring the DUSP-1 mRNA 3′-UTR region fused 3′ prime, PGE2 induced a modest (1.38) increase in promoter (transcriptional) activity while also stabilizing luciferase-DUSP-1 3′UTR mRNA chimeric transcripts (2.6 fold; post-transcriptional regulation). With respect to DUSP-1 protein, PGE2 induced a similar bi-phasic pattern as observed with DUSP-1 mRNA, appearing within 20−30 min, a plateau at 60 min, with protein levels declining to near control levels. A second phase of protein expression was observed from 4 h and DUSP-1 protein was still detectable after 24 h. In terms of measuring DUSP-1 phosphatase activity, PGE2 abrogated rhIl-1β induced phosphorylation of T183/Y185 of SAPK/JNK, a response abolished by knock down of DUSP-1 through targeting by over expression with either a shRNA expression construct or the DUSP-1 inhibitor sanquinarine sulfate. Using specific PGE2 receptor (EP) agonists and antagonists, we observed that the PGE2-dependent